CHAPTER 6

PHARMACEUTICAL APPLICATIONS OF CYCLODEXTRINS

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1 INTRODUCTION

Although cyclodextrins have been known for nearly a century, having been isolated from starch degradation products in 1891 by Villiers <99> and the description of their preparation, isolation and main characteristics having been written between 1903 and 1911 by Schardinger <49 to 51>, it is only during the last ten years that they have provoked real interest in the pharmaceutical domain.

Cyclodextrins are cyclic oligosaccharides containing six, seven or eight glucose units, known as α-, β- and γ-cyclodextrins. Some of their characteristics are given in Table 1. Cyclodextrins are water-soluble, and β-cyclodextrin is the least soluble. Solubility increases sharply with temperature, allowing easy recrystallization by cooling.

Table 1

Characteristics of cyclodextrins

cyclodextrin	number of glucoses	molecular weight	solubility in water (g/100 mℓ)	cavity dimensions (Å)		
				depth	diameter	
					int	ext
α cyclohexa-amylose	6	973	14.50	7.9 to 8.0	4.7 to 5.2	14.6 ±0.4
β cyclohepta-amylose	7	1135	1.85	7.9 to 8.0	6.0 to 6.4	15.4 ±0.4
γ cycloocta-amylose	8	1297	23.30	7.9 to 8.0	7.5 to 8.3	17.5 ±0.4

One of the most interesting properties of cyclodextrins is their ability to form inclusion compounds with a wide variety of poorly water-soluble molecules, which apparently have to satisfy a single condition: to be adaptable entirely, or at least partly, to the cyclodextrin cavity <48>.

The inclusion of a guest molecule in a cyclodextrin constitutes a true molecular microencapsulation that is likely to alter considerably the physicochemical and even biological properties of the guest molecule. This has encouraged pharmacotechnical research, the applications of inclusions being essentially the improvement of molecule stability <6> and, above all, the improvement of their dissolution rate and bioavailability <7>.

2 STABILITY IMPROVEMENT

Stability improvement has three essential objectives: heat stability, resistance to oxidation, and resistance to hydrolysis (or stability in aqueous solution).

2.1 Heat stability

Heat-sensitive substances included in cyclodextrins to improve their stability may be solid or liquid.

2.1.1 *Reduction in volatility*

Reduction in volatility can be demonstrated by a rise in the boiling point or the vaporization conditions (evaporation or sublimation) of products.

Inclusion compounds in β-cyclodextrin with various volatile substances were prepared by Szejtli *et al* <11,67 to 70>. These substances included spices, flavours, essential oils (eucalyptus, peppermint, fennel), anethole, camphor and menthol. The inclusion compounds of aromatic substances contain all the constituents of the original products <68>. Furthermore, the inclusions facilitate the handling of the products, particularly due to the fact that they transform liquids to solids. The value of these inclusions is to enable an improvement in the quality of the pharmaceutical forms in which they are incorporated, especially suppositories <67,70> and inhalations <11,69>. In the case of suppositories, their melting point and hardness are often lowered by adding volatile substances, but the inclusion of these substances in cyclodextrins overcomes these draw-

backs <70>. In the case of inhalations containing high proportions of volatile oils, the preparation is liquid, difficult to handle, and is sometimes volatilized too rapidly if mixed with boiling water. By solidifying the product, inclusion facilitates and slows down its vaporization whilst prolonging its effect <11>.

The volatility of anethole <68> was studied at 70, 80 and 90 °C, under nitrogen, on free anethole, anethole adsorbed on glucose, and the inclusion of anethole in β-cyclodextrin. The evaporated anethole is adsorbed on cooled methanol. Only the inclusion compound resists the increase in temperature and, at 90 °C, the evaporation of anethole remains extremely slight compared with the other two forms (Figure 1).

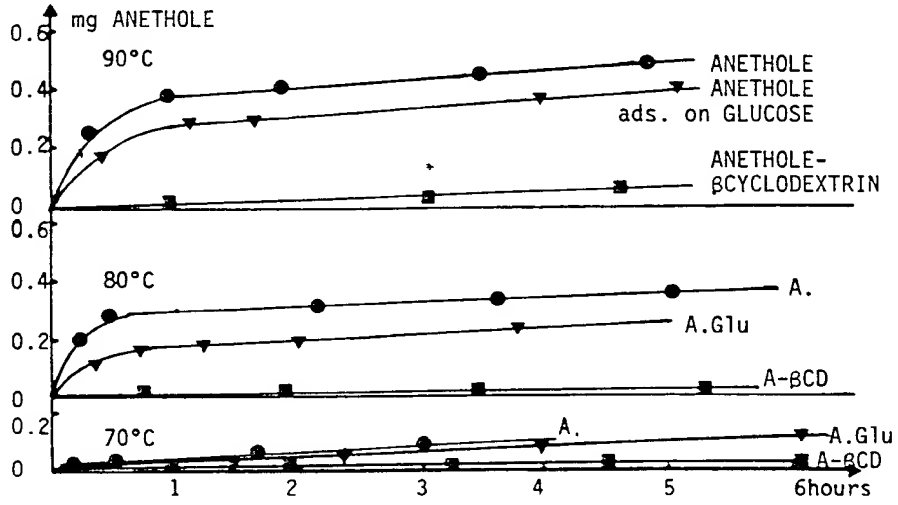

Figure 1 Volatility of anethole at various temperatures as a function of time
(According to <68>, with permission of Acad.Sci.Hung.)

The reduction in volatility of clofibrate, by inclusion in β- and γ-cyclodextrins, has been studied by Uekama *et al* <92> using differential thermal analysis and thermogravimetry (Figure 2). The thermograms of clofibrate show an endothermic peak around 150 °C corresponding to the boiling point: this peak disappears for the inclusion compound. Furthermore, the thermogravimetry curves indicate a significantly reduced volatility of clofibrate resulting from inclusion. Uekama *et al* obtained similar results by the inclusion of cinnamic acid derivatives in β-cyclodextrin <85> and benzaldehyde in α-, β- and γ-cyclodextrins <90>.

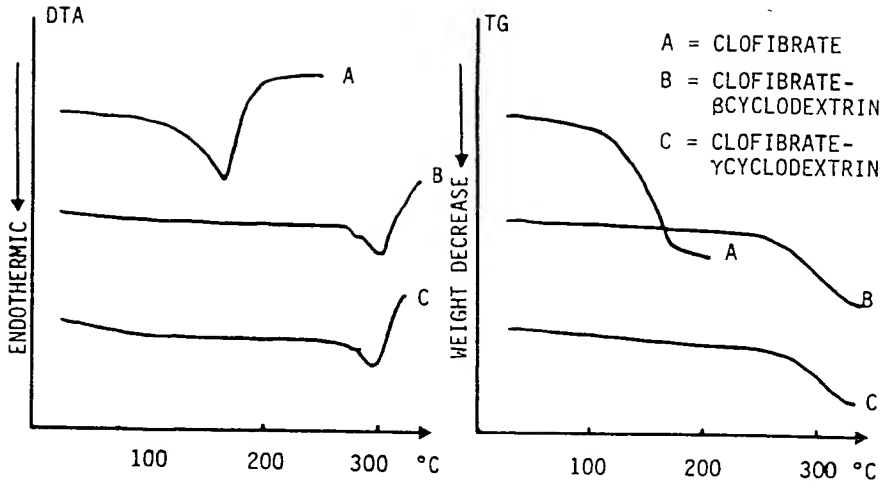

Figure 2 Differential thermal analysis and thermogravimetry of clofibrate and clofibrate β- or γ-cyclodextrin inclusion compounds (According to <92>, with permission of Pharm.Acta.Helv.)

Nakai *et al* <39> used thermogravimetry to study the volatility of the physical mixtures, ground mixtures, inclusion compounds and ground inclusion compounds of parahydroxybenzoic acid and α- and β-cyclodextrins (Table 2).

Table 2

Rate of sublimation of parahydroxybenzoic acid from different systems (According to <39>, with permission of Chem.Pharm.Bull.)

mixture	α-cyclodextrin		β-cyclodextrin	
	180 °C	210 °C	180 °C	210 °C
physical mixture	1.04	1.01	1.07	1.03
ground mixture	0.13	0.59	0.04	0.24
inclusion compound	0	0	0.01	0.10
ground inclusion compound	0.23	0.63	0.07	0.42

The rate given is the percentage (w/w) that sublimed after 30 minutes.

The sublimation of parahydroxybenzoic acid at 180 and 210 °C is considerably reduced by the two inclusion compounds. In the systems with α-cyclodextrin, sublimation is observed with the ground mixture and the ground compound at 180 °C. If the parahydroxybenzoic acid molecules formed strong hydrogen bonds with the hydroxy groups of the α-cyclodextrin molecules, sublimation would be considerably reduced. So it is logical to think that parahydroxybenzoic acid exists in its dimer form in α-cyclodextrin. In the case of the systems with β-cyclodextrin, the ground mixture and ground inclusion show an appreciable reduction in sublimation, although this effect is less than that seen for the inclusion. The inclusion compound with α-cyclodextrin greatly reduces sublimation, most probably due to a tightness constraint, i.e. a more accurate fit within the cavity of the molecule.

Recently Vikmon and Szejtli <97> demonstrated improved stability of Mydeton by inclusion in β-cyclodextrin.

We have seen that the formation of inclusion compounds increases the boiling point and the evaporation or sublimation temperatures of the starting products, and it can also increase their melting point. This has been observed for metronidazole benzoate included in β-cyclodextrin <35> and for prostaglandin $F_{2\alpha}$ included in γ-cyclodextrin <86>.

An interesting case of stabilization achieved by inclusion in β-cyclodextrin is that of the isosorbide 5-mononitrate studied by Uekama *et al* <91>. This is a volatile substance and, during the storage of tablets containing it, needles are formed at the surface, especially if the temperature and humidity conditions are unfavourable. Inclusion eliminates this process and also reduces degradation of the product with time.

2.1.2 *Improvement in heat stability*

A manifestation of an increase in heat resistance is the elevation of the decomposition temperature. Szejtli *et al* <68> investigated a series of essential oils, especially those of marjoram and tarragon. The products to be tested are heated in a test tube whose mouth is connected to a fine capillary tube. A product that discharges water, such as starch, is added into the test tube. The mixture is heated from 60 to 300 °C, increasing the temperature by 10 ±2 °C/min. The evaporation product that leaves by the capillary tube is collected on a thin layer chromatographic plate. With marjoram oil (Figure 3), all the components of the pure product evaporate by 100 °C. This also happens for the physical mixture with β-cyclodextrin: at 240 °C decomposition is observed. On the other hand, with the inclusion compound, the constituents are seen at 160 °C only, and no decomposition is observed up to 300 °C.

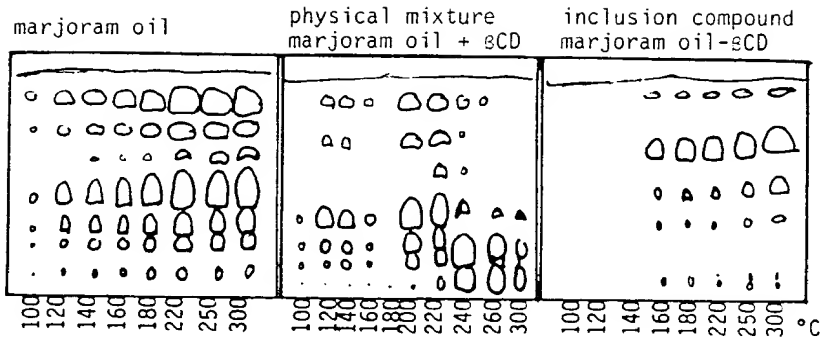

Figure 3 Chromatogram of volatile heat decomposition products of marjoram oil, of a mixture of marjoram oil and β-cyclodextrin, and a marjoram oil β-cyclodextrin inclusion compound (According to <68>, with permission of Acad.Sci.Hung.)

2.2 Oxidation resistance

2.2.1 *Oxygen*

The protective action of inclusion formation with cyclodextrins can be investigated by placing the products to be tested in a Warburg apparatus, under oxygen, at 37 °C. The absorption of oxygen is measured at regular time intervals.

Using this method, Szejtli *et al* <61,62> demonstrated an improvement in the stability of vitamin D_3 when it is included in β-cyclodextrin. From their results, it would appear (Figure 4) that pure vitamin D_3 can fix 140 µℓ/mg of oxygen, and that the physical mixture gives poorer results. On the other hand, the inclusion compound fixes only 11.2% of this amount over the same experimental time period (500 h).

Szejtli <68> uses the same method to study the resistance to oxidation of vegetable essences included in β-cyclodextrin.

2.2.2 *Oxidation accelerators*

Heat, light and metal salts (copper sulphate) all increase the degradation of vitamin D_3 by oxidation. This can be inhibited, or considerably reduced, by inclusion of the vitamin in β-cyclodextrin <61,62> (Figures 5, 6 and 7). The product so treated can be

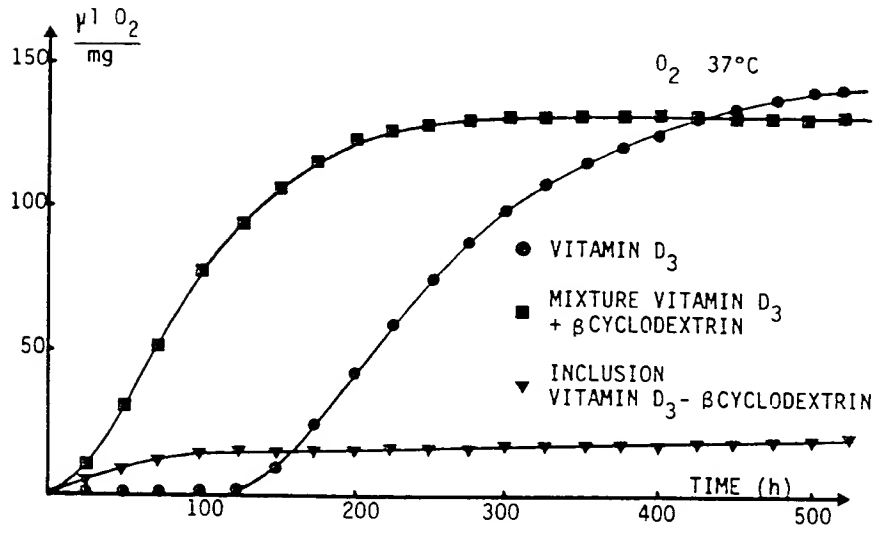

Figure 4 Oxygen uptake of vitamin D_3 and its β-cyclodextrin inclusion compound
(According to <61>, with permission of Die Pharmazie)

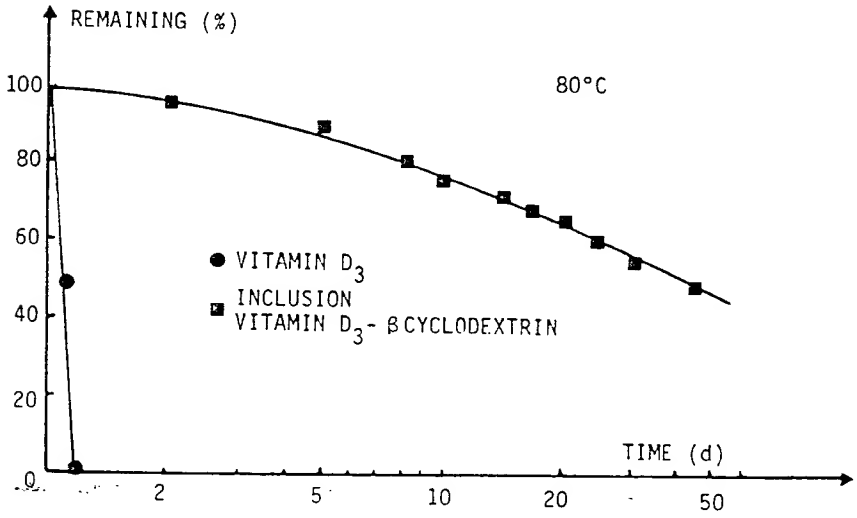

Figure 5 The decomposition of vitamin D_3 at 80 °C in the free state and in inclusion form
(According to <61>, with permission of Die Pharmazie)

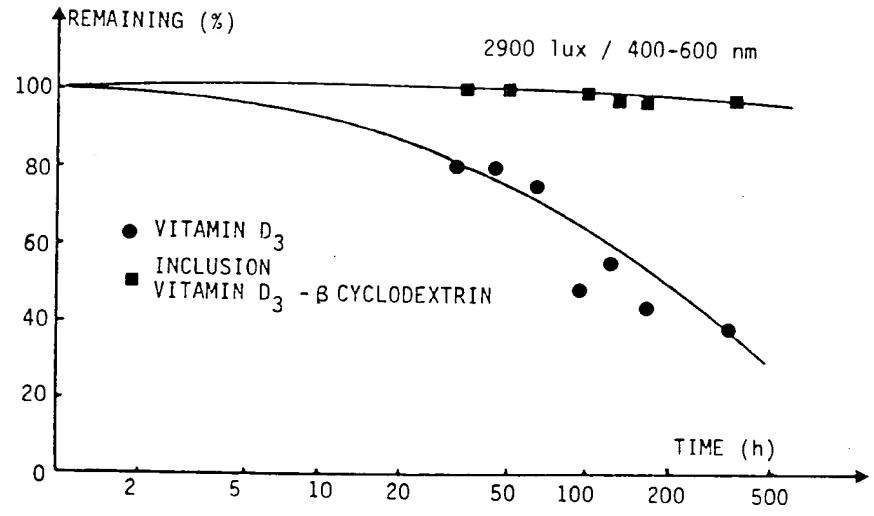

Figure 6 Light-induced decomposition of vitamin D_3 and its β-cyclodextrin inclusion compound
(According to <61>, with permission of Die Pharmazie)

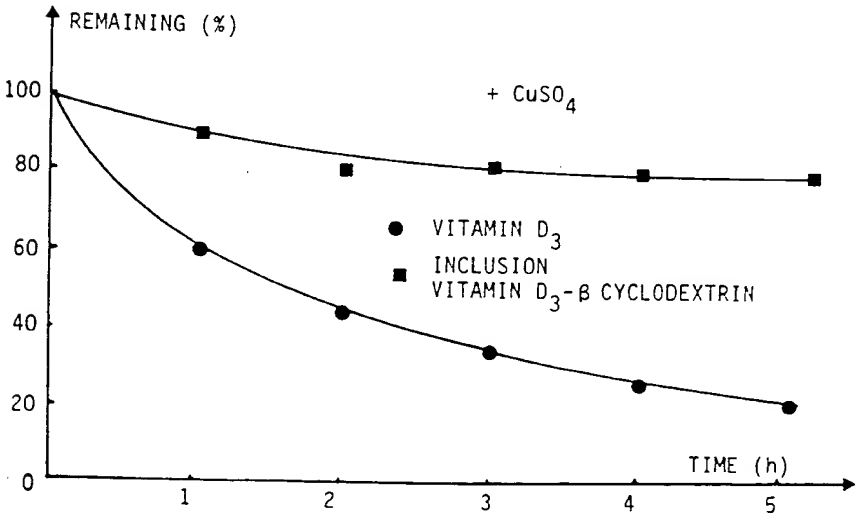

Figure 7 Stability of vitamin D_3 and its β-cyclodextrin inclusion compound against cupric sulphate
(According to <61>, with permission of Die Pharmazie)

presented in tablet form, having better stability against heat than tablets of the pure vitamin <61,62>. The vitamin D_3 β-cyclodextrin inclusion compound preserves 94% of its therapeutic activity, even after being stored for seven days at 60 °C <56>.

Similarly, the inclusion of vitamin A in α-cyclodextrin increases its stability against heat <28>.

The sensitivity to light of clofibrate <92> and guaiazulene <100> is reduced by inclusion in β- and γ-cyclodextrins.

2.3 Resistance to hydrolysis and to degradation in solution

The above results suggest that the inclusion of a guest molecule in cyclodextrins confers good protection to the guest molecule. But this is not always the case for hydrolysis, where the results are much more equivocal.

2.3.1 *Vitamin K_3*

In some cases, depending on the pH, inclusion can render the active ingredients unstable in aqueous solution.

Szejtli <64> noted that the inclusion of vitamin K_3 in β-cyclodextrin (which improves its heat stability in the solid state <65>) has no effect on its light stability in neutral or slightly acidic solution. Furthermore, at basic pH values, decomposition is promoted by β-cyclodextrin (Figure 8).

2.3.2 *Steroids*

Møllgaard Andersen and Bundgaard <34> have shown that the rate of degradation of hydrocortisone increases threefold in the presence of β-cyclodextrin in alkaline aqueous solution, but that inclusion compound formation has no action on the steroid in neutral or acid solution. The experiment is conducted in the presence of sodium edetate to reduce the catalytic effect of traces of metallic impurities. The authors explain their results by the mode of degradation of hydrocortisone: in acid aqueous solution, hydrocortisone is chiefly degradated to the glyoxal 17-deoxy derivative by elimination of the water molecule by initial enolization of the keto group on C_{20}.

The same non-oxidative reaction occurs in neutral solution in the presence of sodium edetate. But in an alkaline solution containing the same sequestering agent, a 17-ketosteroid and a 17-deoxy-20-

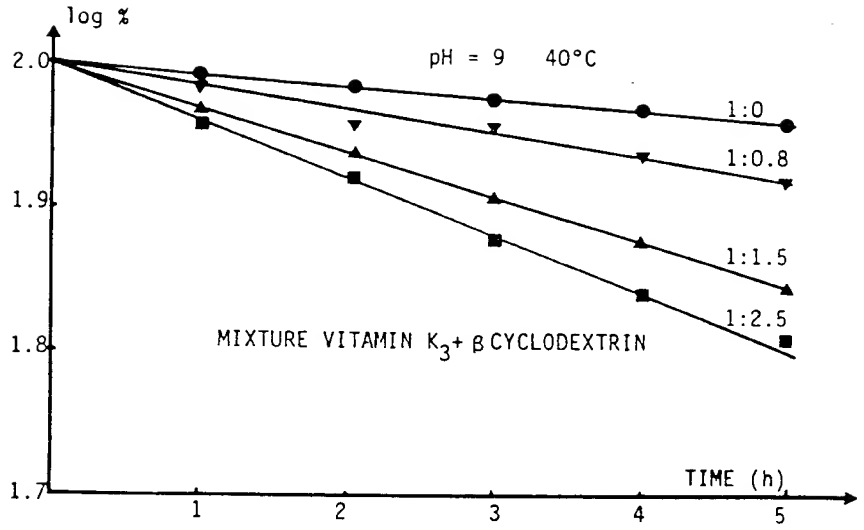

Figure 8 β-cyclodextrin enhances the decomposition of vitamin K_3 in alkaline solutions
(According to <64>, with permission of Die Pharmazie)

hydroxy-21-acid derivative are the main degradation products. In all these reactions, enolization of the lateral dehydroxyacetone chain on C_{17} may be considered to be the first stage in the degradation process. In acid solution, the enolization is the determining stage, but in alkaline solution the enolization is reversible and not determinant. Thus the accelerating action of β-cyclodextrin in alkaline solution may be attributed to a displacement of the keto/enol equilibrium towards the more reactive enol form by β-cyclodextrin.

These authors <1> investigated the stability of betamethasone 17-valerate in aqueous alkaline solution, in which this substance undergoes a rearrangement into the less active 21-valerate. While α-cyclodextrin has no effect on this rearrangement, β-cyclodextrin accelerates it, and γ-cyclodextrin slows it down substantially. These results are explained by the differences of conformation of the inclusion compounds (1:1) formed.

2.3.3 *Nitrazepam*

Studying nitrazepam, Møllgaard Andersen and Bundgaard <32> showed that the presence of β-cyclodextrin does not have a significant influence on the rate of hydrolysis of this product in 0.1 M hydrochloric acid

solution. This may be either because included diazepam has the same reactivity as that of the non-included product, or because it does not form an inclusion compound in acid solution. The pKa of nitrazepam is 2.8, so in reaction solution the product would be entirely in the protonic form. In general, non-ionized molecules more easily form inclusion compounds with cyclodextrins than ionized molecules.

2.3.4 *Aspirin*

The effect on stability of the inclusion of aspirin has been dealt with in several publications by Terada, Yamamoto and Nakai. Terada *et al* <72>, studying the stability of aspirin in the solid state, either pure or associated with α-, β- and γ-cyclodextrins in physical mixtures or ground mixtures, arrived at different conclusions depending on the type of the association and the nature of the cyclodextrin (Figure 9). At 40 °C and 32.3% relative humidity, pure aspirin is relatively stable for two months. The physical mixtures are also nearly as stable, and the inclusion compounds (possible only with β- and γ-cyclodextrins) are almost stable in the case of β-cyclodextrin, and unstable with γ-cyclodextrin. All the ground associations (mixtures and inclusions) were unstable. In conclusion, the association of aspirin in the solid state with cyclodextrin is never favourable, and may even be detrimental. The decomposition rate is related to the presumed state of the acetoxyl groups of aspirin in

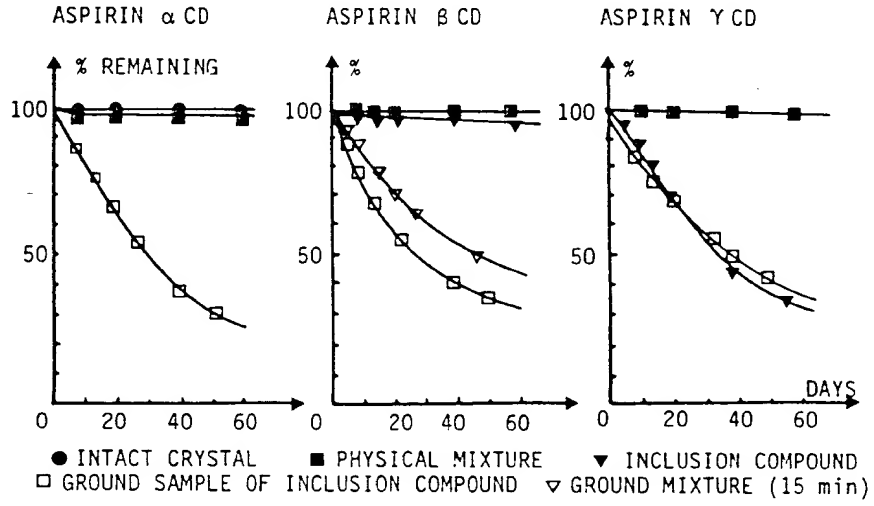

Figure 9 Solid state aspirin decomposition in aspirin and cyclodextrin systems at 40 °C and 32.3% relative humidity (According to <72> with permission from Y. Nakai)

cyclodextrin. When the acetoxyl groups are in a free state, the aspirin molecules are relatively stable. On the other hand, when the acetoxyl groups are linked by hydrogen bonds with the hydroxyl groups of the cyclodextrins, the aspirin molecule becomes unstable.

Nakai <39>, studying the hydrolysis of aspirin in pH 1 acid solution in the presence of α-, β- and γ-cyclodextrins, shows the first-order degradation kinetics, whose constants are given in Table 3.

Table 3

Constants of the rate of degradation of aspirin at pH 1
(According to <39>, with permission of Chem.Pharm.Bull.)

	degradation rate constant at				
	15 °C	25 °C	35 °C	45 °C	55 °C
control (without cyclodextrin)	0.0760	0.200	0.536	1.17	2.86
with α-cyclodextrin	0.0759	0.206	0.528	1.16	2.83
with β-cyclodextrin	0.0493	0.150	0.450	1.09	2.74
with γ-cyclodextrin	0.0703	0.190	0.524	1.16	2.88

Although the constant of degradation does not show any significant variations in the presence of α-cyclodextrin, a reduction is observed in the presence of β-cyclodextrin at all the temperatures studied. The addition of γ-cyclodextrin results in a reduction in the degradation constant, but much less marked than in the case of β-cyclodextrin. Although these results are in line with those obtained for the solid state <72>, they have been interpreted differently. Hydrolysis under acid conditions is accelerated by the addition of a proton to the oxygen molecule of the carbonyl group, and the nucleophilic attack by the water molecule on the carbon of the carbonyl group is blocked, so the direct action of water on hydrolysis is negligible. The pKa of β-cyclodextrin is 12 and, under these conditions, it is entirely in the molecular state at pH 1. From an NMR study it is assumed that the aspirin molecule is included in the cavity of β-cyclodextrin, and because of this, it is difficult for a proton or a water molecule to approach the acetoxyl carbonyl group of the included aspirin molecule. On the other hand, the addition of α-cyclodextrin does not change the hydrolysis of aspirin. This is in concordance with an NMR study which shows that aspirin molecules are not included in the cavity of α-cyclodextrin in aqueous solution. γ-cyclodextrin has only a feeble hydrolysis inhibiting effect which may be attributed to the large empty space into which a proton or water molecule can penetrate.

2.3.5 *Indomethacin and non-steroidal anti-inflammatory agents*

The results of the effect of inclusion of indomethacin in β-cyclodextrin are contradictory. Szejtli <66> described this inclusion and showed that, in aqueous solution at pH 8, stored for two hours at 37 and 60 °C, decomposition is the same for the pure product and the inclusion. In a patent document, Sumitomo Chemical <59> reported a stabilization of indomethacin by inclusion in β-cyclodextrin. Hamada <12> confirmed this stabilization with β-cyclodextrin, whereas the addition of α-cyclodextrin or glucose accelerates hydrolysis. This result is explicable by the size of the functional group to be included in the cyclodextrin. This is possible with β-cyclodextrin and not possible with α-cyclodextrin.

Hamada <12> studied not only indomethacin but a whole series of non-steroidal anti-inflammatory agents, and compared their stability in aqueous solution, in the pure state, or after addition of α-cyclodextrin, β-cyclodextrin or glucose. After a certain time lag, β-cyclodextrin increases the degradation of azapropazone at pH 6. The same phenomenon occurs at pH 8 without a time lag. With phenylbutazone, degradation at pH 5.5 is inhibited by β-cyclodextrin (Figure 10). At pH 8, phenylbutazone is so stable that β-cyclodextrin has no action, α-cyclodextrin has the same effect as β-cyclodextrin, but glucose has a deleterious action. Finally, although β-cyclodextrin inhibits the hydrolysis of indomethacin, it is slightly increased by α-cyclo-

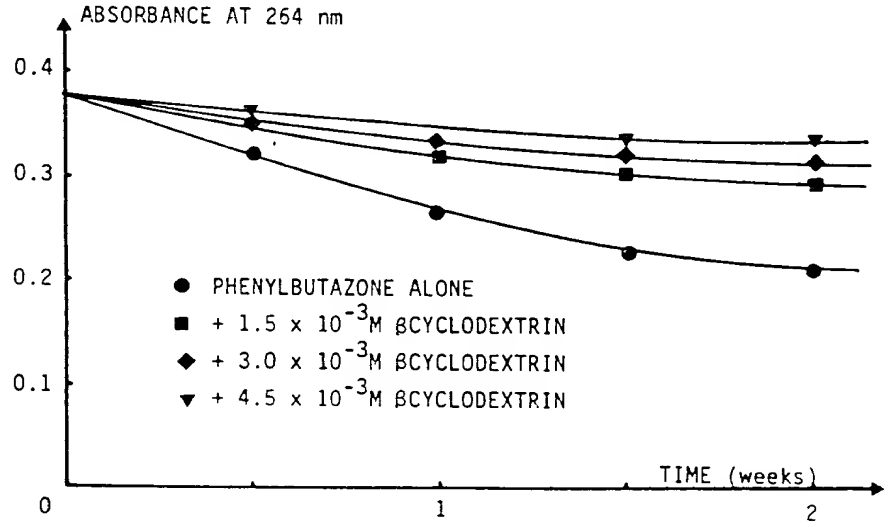

Figure 10 Effect of β-cyclodextrin on the stability of $2 \cdot 10^{-5}$ mol/ℓ phenylbutazone aqueous solution at pH 5.5 and 30 °C (According to <12>, with permission of Chem.Pharm.Bull.)

dextrin. This is probably due to the fact that α-cyclodextrin cannot include indomethacin. Even so, despite the capacity of a cyclodextrin to include a guest molecule, the result may be either an increase or a decrease in stability.

2.3.6 *Barbiturates*

Barbiturates have also formed the subject of various studies. Nagai <38>, noting the extreme instability of hexobarbital in aqueous solution, looked for a protective action with a series of products, including α-, β- and γ-cyclodextrins. The rate of degradation of hexobarbital in aqueous alkaline solution at pH 12 (phosphate/sodium hydroxide buffer) was slightly increased by β-cyclodextrin and slightly decreased by α- and γ-cyclodextrins (Table 4).

Table 4

Percentage residual hexobarbital in buffer solution at pH 12 in the presence of cyclodextrins (According to <38>, with permission of T. Nagai)

additive	time (h)						
	0	1	2	3	4	8	12
without	100	80.8	68.5	57.1	49.5	27.0	11.3
α-cyclodextrin	100	84.7	73.4	61.2	54.0	35.3	-
β-cyclodextrin	100	77.9	60.5	-	37.8	14.9	6.1
γ-cyclodextrin	100	81.2	71.8	60.6	56.8	29.9	15.3

Min <31> and Koizumi <23,26> report an improvement in the stability of phenobarbital in aqueous solution and various barbiturates after the addition of β-cyclodextrin.

2.3.7 *Bencyclane fumarate*

This anticonvulsive and vasodilating agent, unstable in acid medium, was studied by Fujioka *et al* <10>. Inclusions with cyclodextrins were prepared and isolated by freeze-drying. Their stability was tested in 0.1 N HCl acid medium at 37 °C. In all the cases tested, the degradation kinetics for bencyclane fumarate were apparently monoexponential. The cyclodextrins slow down this degradation and their effect increases in the order α-, γ- and β-cyclodextrin. The constant for the rate of degradation as measured from the slope of the

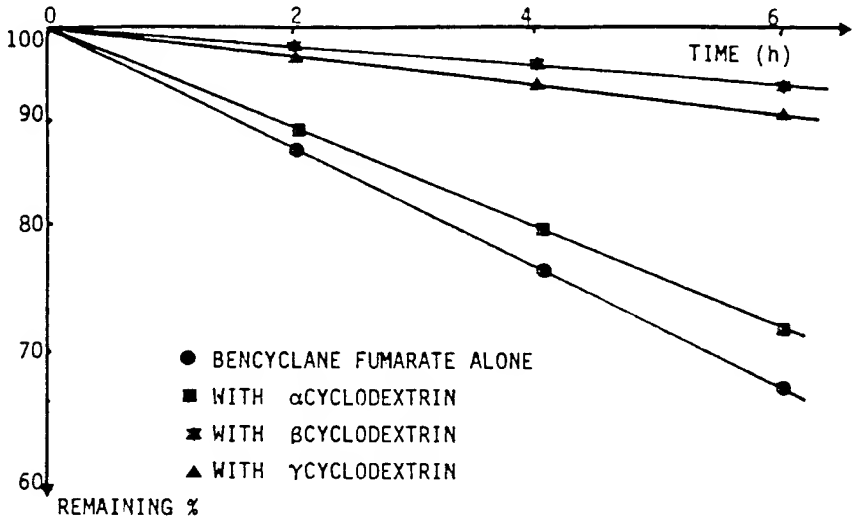

Figure 11 Stability of bencyclane fumarate in 0.1 N HCl solution at 37 °C
(According to <10>, with permission of Chem.Pharm.Bull.)

degradation curves (Figure 11) falls in the order pure product, inclusions with α-, then γ- and then β-cyclodextrin. This delaying effect may in part be attributed to the differences between the constants of formation of the three compounds. Thus the inclusion with α-cyclodextrin which, on X-ray diffraction, shows an incomplete transformation in the amorphous state, has the weakest stabilizing effect. It should also be pointed out that the inclusion of this product reduces its bitter taste.

2.3.8 *Proscillaridin*

Proscillaridin has been studied by Uekama <82>. It is a cardiotonic glucoside which has a poor bioavailability by the oral route, probably due to its poor solubility and its instability in the gastro-intestinal juices. Inclusions with α-, β- and γ-cyclodextrins produce a reduction in the instability at pH 1.46 and 37 °C. However, this result is only significant with β- and γ-cyclodextrins (Figure 12). This suggests that the cavity of α-cyclodextrin, the smallest of the three, does not protect the proscillaridin molecule against acid hydrolysis.

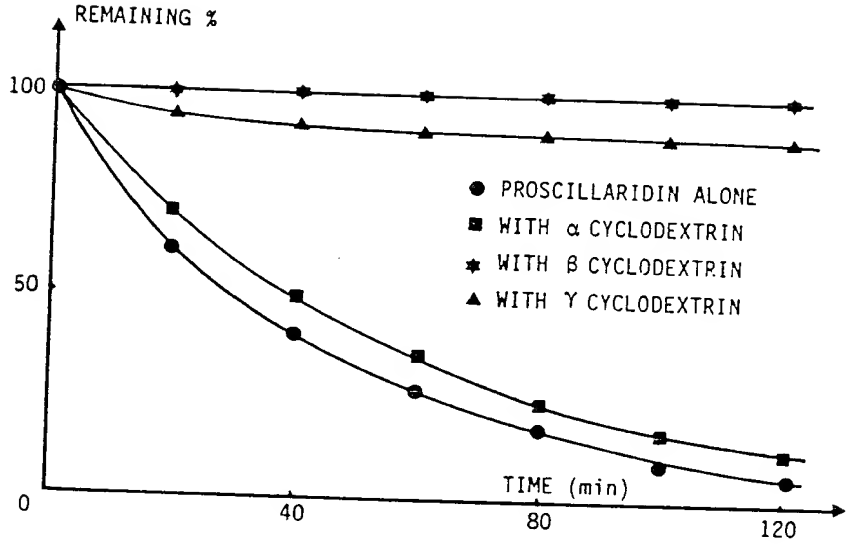

Figure 12 Degradation of proscillaridin in the absence and presence of cyclodextrins in KCl buffer (pH 1.46) at 37 °C (According to <82>, with permission of Acta Pharm.Suec.)

2.3.9 *Metronidazole benzoate*

This drug was studied by Møllgaard Andersen and Bundgaard <35>, who found that inclusion of metronidazole benzoate resulted in two kinds of protective effect. First there is a slowdown in the hydrolysis rate, and secondly there is a slowing down of particle growth in suspension.

When the product is hydrolysed at pH 10.4 (0.05 M carbonate buffer) at 24 °C, the degradation of metronidazole always occurs in line with first-order kinetics. The constant of hydrolysis falls in an asymptotic manner when the concentration of β-cyclodextrin increased (Figure 13). This stabilizing action against hydrolysis suggests that the inclusion of the ester in the cyclodextrin cavity occurs in such a manner that the carbonyl ester group is partially protected against attack by the hydroxide or alkoxide ions of the cyclodextrin. As benzoic acid is known to form inclusion compounds with cyclodextrins, it is probably the benzoate half of the metronidazole ester which is implicated in the formation of the inclusion.

When anhydrous metronidazole benzoate is dispersed in aqueous solution, there is a very marked increase in the size of its particles if the suspension is stored for a few weeks at low temperature (around 8 °C).

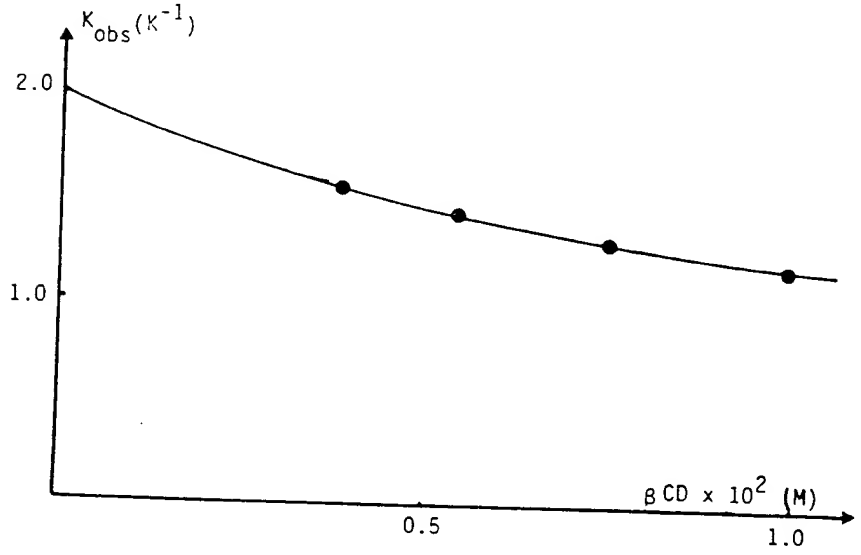

Figure 13 The effect of β-cyclodextrin concentration on the constant of hydrolysis of metronidazole benzoate at 24 °C in 0.05 M carbonate buffer solution at pH 10.4 (According to <35>, with permission of Int.J.Pharm.)

This crystal growth is due to the transitional phase between the anhydrous form and the monohydrate form. Inclusion in β-cyclodextrin reduces this transitional phase and thus increases the physical stability of the suspension.

2.3.10 *Prostaglandins*

Numerous patent documents, in particular of Japanese origin, relate to the inclusion of prostaglandins in α- and β-cyclodextrins, or their methylated derivatives <13,14,36,37,43,71>. Interpretation of these results is often difficult. Nevertheless, in general, there is an increase in the stability of aqueous solutions, and ageing of freeze-dried products is also improved.

Uekama <84> showed that the inclusion of prostaglandin E_1 in γ-cyclodextrin increases its heat stability and slows down its conversion to prostaglandin A_1.

2.3.11 *Antibiotics*

Various antibiotics have been studied, such as ampicillin and methycillin with β-cyclodextrin <17>, and amphotericin-B with γ-cyclodextrin <47>. In each case, the cyclodextrin seems to be a good agent to slow down the hydrolysis rate of the antibiotic for various pH.

2.3.12 *Cinnarizine*

Tokumura <74> studied inclusions of cinnarizine with β-cyclodextrin prepared by the coprecipitation, neutralization and spray-drying methods. For these last two methods, it is necessary to dissolve the cinnarizine in an acidic solution in which degradation may occur. In fact the presence of β-cyclodextrin prevents this degradation.

3 IMPROVEMENT IN DISSOLUTION AND BIOAVAILABILITY

3.1 *In vitro* investigations: higher water solubility

3.1.1 *Demonstration of the effect of cyclodextrins*

Hamada <12> studied a series of non-steroidal anti-inflammatory substances (azapropazone, indomethacin, flufenamic acid and phenylbutazone) and compared the rôle of the addition of glucose and α- and β-cyclodextrins on water solubility. Glucose appears to have no effect, α-cyclodextrin is either ineffective or only slightly effective, and β-cyclodextrin causes an increase in solubility. The case of flufenamic acid is shown in Figure 14. These results are explained by the formation of inclusion compounds which depends on the size of the guest molecule compared with the internal diameter of the cyclodextrin. It depends also on the possibility of interaction between host and guest molecules.

It is unnecessary for the inclusion to be preformed for higher solubility to occur. Corrigan and Stanley <3> worked on phenobarbitone and β-cyclodextrin, presented either as the physical mixture or as a freeze-dried mixture. In both cases a fourfold increase in phenobarbitone solubility is observed.

These authors <4>, studying benzothiazide derivatives with β-cyclodextrin, showed that, if the dissolution rate is higher for the physical mixture than for the active ingredient alone, it is still lower than that of the freeze-dried mixture (Table 5). This can be

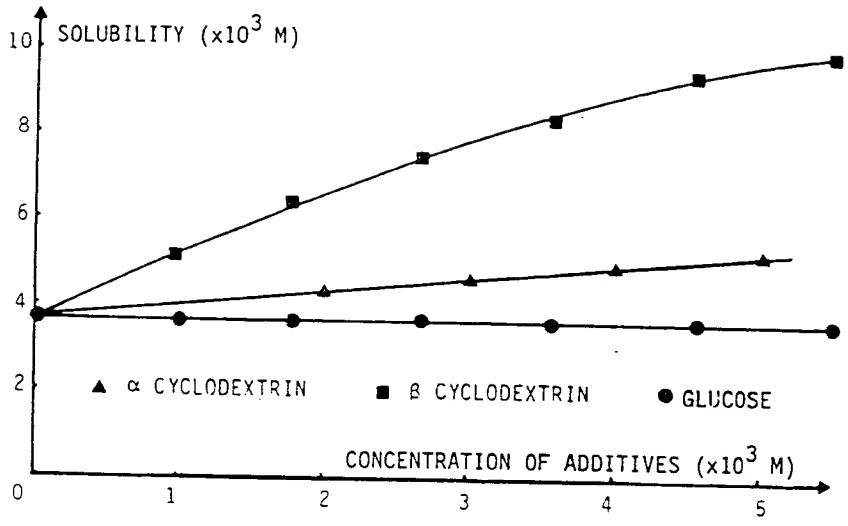

Figure 14 Effects of α-cyclodextrin, β-cyclodextrin and glucose on the solubility of flufenamic acid at 35 °C (According to <12>, with permission of Chem.Pharm.Bull.)

Table 5

Initial dissolution rate of benzothiazide derivatives alone or in equimolecular mixtures with β-cyclodextrin (According to <4>, with permission of J.Pharm.Pharmacol. and the author, O.I. Corrigan)

	dissolution rate $(mol/\ell \cdot cm^{-2} \cdot h^{-1}) \times 10^3$		
	pure	freeze-dried mixture	physical mixture
bendrofluazide	0.43	25.75	17.2
chlorothiazide	7.78	64.79	44.6
hydrochlorothiazide	15.28	56.15	47.1
hydroflumethiazide	8.51	27.83	25.8

explained either by the hydrophily of the freeze-dried products and their amorphous character, or by the existence in the freeze-dried mixture of a varying proportion of preformed inclusion compound.

3.1.2 *Solubility diagram and stability constant*

Uekama *et al* applied Higuchi's solubility analysis to various products, such as digitoxin <79>, digoxin <81>, eighteen steroidal hormones <80>, proscillaridin <82>, spironolactone <53>, clofibrate <92>, and flurbiprofen <45>, all associated with α-, β- and γ-cyclodextrins.

Depending on the cyclodextrin employed, various types of diagram can be obtained with the above-mentioned products. For example, for spironolactone <53> (Figure 15) with α-cyclodextrin, solubility increases linearly with cyclodextrin concentration. The curve is of Higuchi's type A_L and corresponds to the formation of an inclusion compound with the stoichiometry 1:1. With β- and γ-cyclodextrins, the curves are of Higuchi's type B_S: after a linear rise, a plateau is observed, followed by a decrease corresponding to the precipitation of a microcrystalline inclusion compound with a different stoichiometry. In the case of flurbiprofen <45>, the curve obtained with α-cyclodextrin, of Higuchi's type A , is not linear but slightly incurved toward the top, indicating the existence in solution of inclusion compounds of a stoichiometry higher than 1:1.

Diagrams of the same type are obtained by Uekama *et al* for propylparaben with β-cyclodextrin <96>, and for prostaglandins E_1 <85> and $F_{2\alpha}$ <87> with γ-cyclodextrin, and by Møllgaard Andersen <34> for hydrocortisone with β-cyclodextrin.

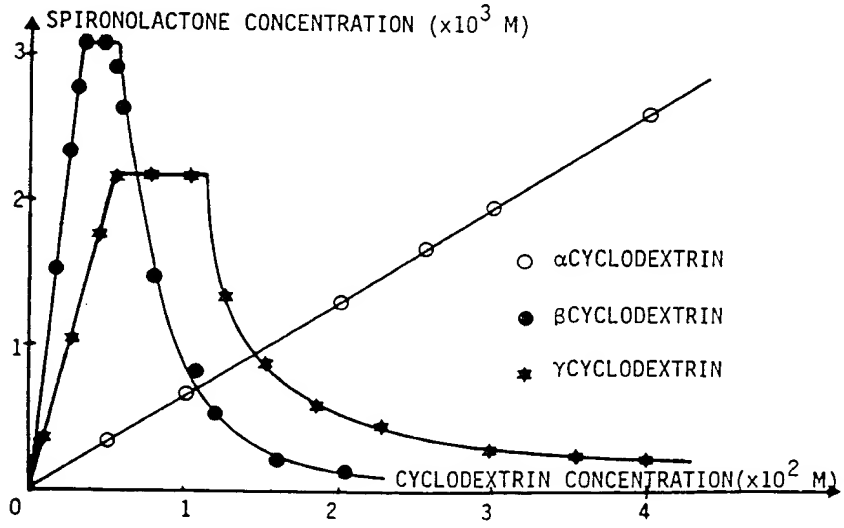

Figure 15 Phase-solubility diagram of spironolactone cyclodextrin systems at 25 °C
(According to <53>, with permission of Chem.Pharm.Bull.)

Phenytoin <77> seems to lead only to curves of the A_L type, regardless of the cyclodextrin used. In some cases, without staying linear, the curves do not present a plateau before the appearance of a decrease <23,27>. With spironolactone in particular, Møllgaard Andersen <33> does not reproduce the curves obtained by Seo and Uekama <53>, and emphasizes the importance of the quantities of spironolactone employed to establish this diagram.

The plotting of such diagrams serves to calculate an apparent stability constant from the straight part of the curves A_L or B_S:

$$K = \frac{S}{C_s(1 - S)}$$

where:

- S slope of the curve representing solubility as a function of cyclodextrin concentration,
- C_s solubility of the product investigated, without cyclodextrin, e.g. intercept of the curve with the axis of solubilities.

The stability constant reflects the correct adjustment of the guest molecule inside the cavity of the cyclodextrin host molecule. For example, the stability constants calculated by Seo and Uekama <53> for the inclusion compound of spironolactone with α-, β- and γ-cyclodextrins are respectively 960, 27,500 and 7600 mol^{-1}. As a rule, steroids display better interaction with β- or γ-cyclodextrins, since α-cyclodextrin is much too small to allow inclusion (Table 6) <80>.

Table 6

Apparent stability constants of steroids with α-, β- and γ-cyclodextrins (According to <80>, with permission of Int.J.Pharm.)

product	K_α	K_β	K_γ
hydrocortisone	57	1,720	2,240
cortisone	63	2,300	2,170
hydrocortisone acetate	88	3,250	2,270
cortisone acetate	86	4,150	2,470
progesterone	145	13,300	24,000
testosterone	134	7,540	16,500
prednisolone	298	3,600	3,240
prednisolone acetate	274	5,770	3,880
triamcinolone	121	2,370	9,920
triamcinolone acetonide	256	3,230	26,100
triamcinolone diacetate	300	3,530	12,100
dexamethazone	169	4,660	26,600
betamethasone	223	5,420	21,600
betamethasone acetate	316	9,560	37,300
betamethasone-17 valerate	302	2,990	9,850
paramethazone	489	2,540	8,310
fluocinolone acetonide	297	3,000	31,900
beclomethazone dipropionate	354	1,120	6,300

In the case of benzodiazepines, the results are of the same type: stability constants are higher with β- and γ-cyclodextrins, but their value is generally lower than that of steroids, indicating a poor adjustment of the products (Table 7) <89>.

Table 7

Apparent stability constants of benzodiazepines with α-, β- and γ-cyclodextrins (According to <89>, with permission from Int.J.Pharm.)

product	K_α	K_β	K_γ
diazepam	24	220	120
medazepam	46	260	160
fludiazepam	27	220	190
nitrazepam	26	96	33
nimetazepam	24	55	38
flunitrazepam	21	77	40
clonazepam	22	80	58
flurazepam	10	120	130
lorazepam	27	320	140
oxazepam	44	170	45
bromazepam	60	55	31
clobazepam	10	49	36
chlordiazepoxide	44	23	140

Stability constants with β-cyclodextrin calculated by Møllgaard Andersen and Bundgaard <32> are of the same order except for medazepam, which is exceptionally high (Table 8). These authors explain their results by the effects of its high lipophilicity.

Table 8

Apparent stability constants of benzodiazepines with β-cyclodextrin (According to <32>, with permission from Arch.Pharm.Chem., Sci.Ed.)

product	K_β	K_α	K_γ
diazepam	210	25	95
medazepam	1,790	240	325
nitrazepam	130		
flunitrazepam	65		
clonazepam	80		
lorazepam	225		
oxazepam	185		
bromazepam	90		
dimethyldiazepam	230		
chlordiazepoxide	550		

3.1.3 *Dissolution of inclusion compounds*

Solubility diagrams remain too theoretical for practical application, because they are plotted when equilibrium is reached, in other words after four or ten days of stirring.

The analysis of the dissolution kinetics of solid inclusion compounds is often preferable, because this can be used to reveal not only an improvement in solubility, but also the rate of passage into solution. This method is very often used for solid dispersions (dissolution and drying) of active ingredients and cyclodextrin <4,22,52>, physical mixtures <4,22,62,66>, or inclusions themselves <4,19,33,46,53,62,65, 66,79 to 82,84,86,87,92,94>.

These studies point out the value of using a solid inclusion from the galenic standpoint, rather than the simple physical mixture <47,65,66>, or a freeze-dried or spray-dried product in other cases <20,21>. Kata <22>, studying the rate of dissolution of products containing vinpocetine base and γ-cyclodextrin, prepared by various methods (kneading, co-pulverization, co-pulverization + Tween 20, spray embedding, and physical mixture), concludes that not only is the preparation method important, but also the percentage composition of the products and the presence of tenside. Vikmon <98> demonstrated the value of γ-cyclodextrin in the obtention of a true solution of amphotericin-B, compared with the obtention of a colloidal dispersion in aqueous medium by using sodium lauryl sulphate or sodium deoxycholate.

A comparison of the inclusion compounds obtained with different cyclodextrins is also interesting. With clofibrate <92>, the obtention of a solid inclusion is not possible with α-cyclodextrin whose solubility diagram is of the type A_L. On the other hand, β- and γ-cyclodextrins lead to solid inclusion compounds (curves B_S) with stability constants of 1550 and 110 respectively, indicating a better molecular adjustment with β-cyclodextrin than with γ-cyclodextrin. Dissolution studies reveal that the highest dissolution rate is obtained with γ-cyclodextrin (Figure 16). The authors explained this result by a lower crystallinity of the inclusion compound obtained with γ-cyclodextrin as shown by the X-ray analysis. A comparable result is obtained by Otagiri and Uekama <45> with flurbiprofen. With β- and γ-cyclodextrins, this product gives solid inclusion compounds displaying stability constants of 5100 and 460 mol^{-1} respectively, and the crystallinity of the inclusion in γ-cyclodextrin is less pronounced than that of the inclusion in β-cyclodextrin. The results of the dissolution of these substances reveal not only faster dissolution for the γ-cyclodextrin inclusion compound, but also a progressive dissociation of this compound in aqueous medium, rapidly causing precipitation of free flurbiprofen <46> (Figure 17).

For the more convenient study of these dissociation mechanisms, rather than using the dissolution method consisting of dispersing a quantity of test product in the dissolution medium, it is often more interesting to use the rotary disc method. This method offers the advantage of linearizing the dissolution curves when dissolution is uniform, but,

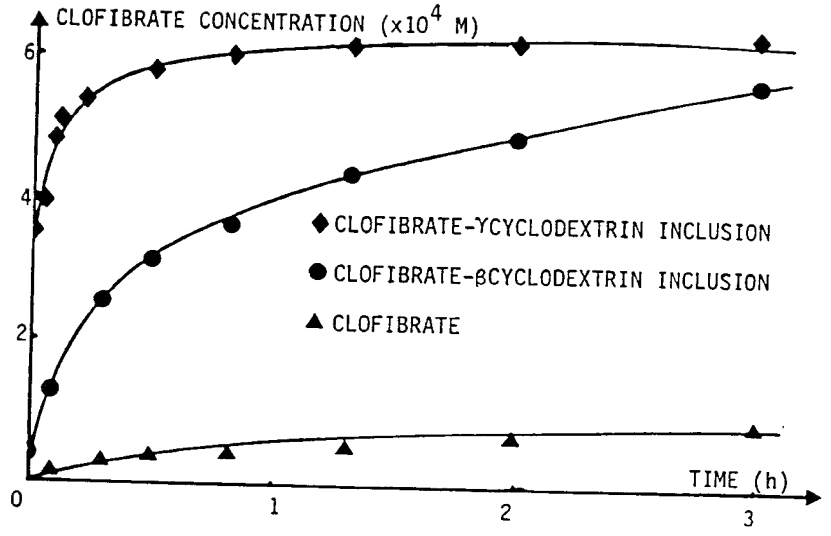

Figure 16 Dissolution of clofibrate and its cyclodextrin inclusion compounds in water at 25 °C (According to <92>, with permission of Pharm.Acta Helv.)

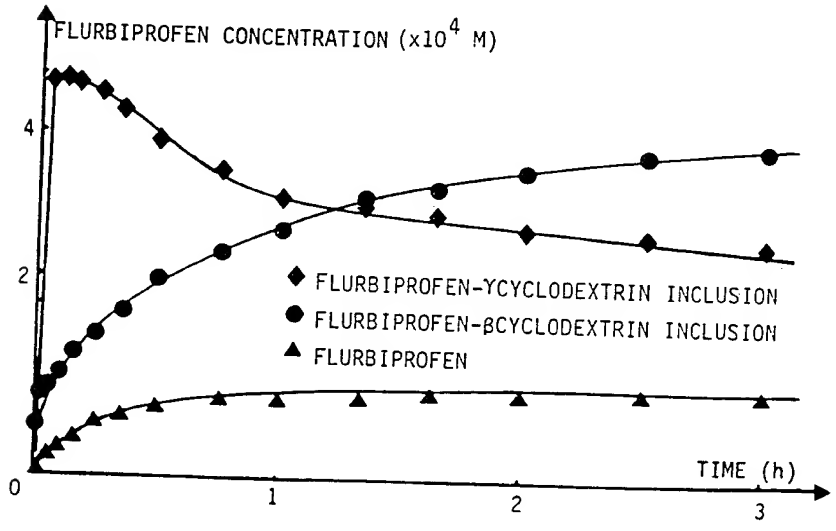

Figure 17 Dissolution of flurbiprofen and its cyclodextrin inclusion compounds in water at 25 °C (According to <46>, with permission of Acta Pharm.Suec.)

on the other hand, if the inclusion compound decomposes in aqueous medium, the released active ingredient reprecipitates, and the curve obtained by the rotary disc method displays a negative curve versus time. Because it offers a particularly clear representation, this method has often been used <33,46,53,80,81,82,87>.

3.1.4 *Diffusion through semi-permeable membranes*

The foregoing studies help to establish a hypothesis according to which water-soluble inclusion compounds, by dissociation, increase the bioavailability of the active ingredients they contain, a hypothesis which needs to be substantiated.

This is why Szejtli <59> and Uekama <46,53,88,89,92,94> investigated the possibilities of the diffusion of active ingredients or their inclusion compounds through semi-permeable membranes. To do this, they employed systems comprising a cellophane membrane between a donor compartment and an acceptor compartment, each equipped with a stirring system.

With the donor and acceptor compartments filled with water, and the test products (active ingredient alone and inclusion compound) added in the solid state in the donor compartment, the diffusion of the inclusion compound is often not as significant as expected. In some cases, such as fendiline in β-cyclodextrin <58>, it is lower than that of the active ingredient alone, and in other cases, such as that of flurbiprofen in β- and γ-cyclodextrins <46>, although it is better than that of the active ingredient alone, it does not agree with the comparative effect of those of cyclodextrins on dissolution (Figure 18).

To explain this result, Uekama <46> compared it with that obtained by placing the solutions of active ingredient and inclusion compound directly in the donor compartment. In this case, the active ingredient (flurbiprofen) diffuses better than the inclusion compound (Figure 19). Diffusion is closely dependent on molecular size, and the inclusion compounds diffuse with greater difficulty than the guest molecule. In addition, diffusion must be related to the stability constant: the higher the constant the less the diffusion (for inclusion compounds 1:1 of flurbiprofen, K_β = 5100 mol^{-1}, and K_γ = 460 mol^{-1}). Consequently, diffusion through cellophane membranes is strongly dependent on molecular size, and, if the products are previously dissolved, the diffusion of the inclusion compound is slower than diffusion of the active ingredient alone. On the other hand, if the products are introduced in the solid state, the high rate of dissolution of the inclusion compound, compared with that of the active ingredient alone, enables it to diffuse more rapidly than the active ingredient.

These studies demonstrate that this investigative method is perhaps not a good indication of possible absorption *in vitro*.

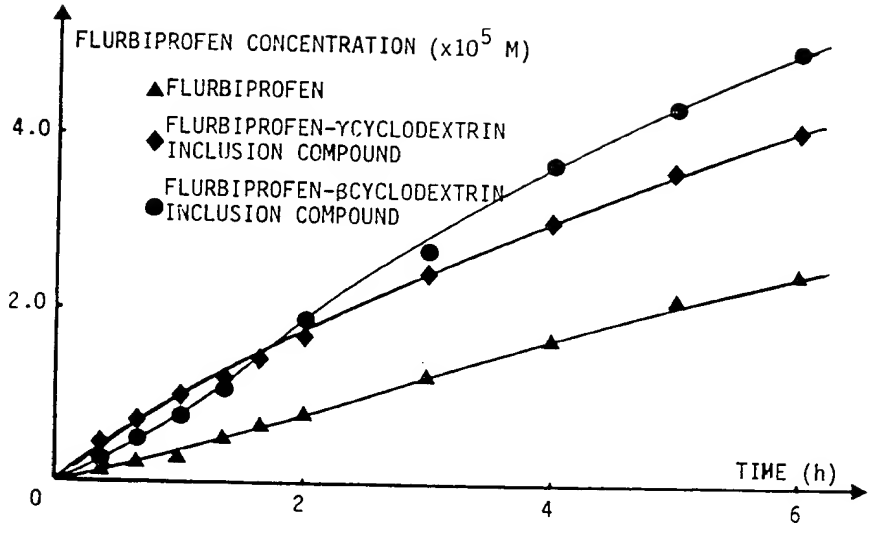

Figure 18 Permeation of flurbiprofen and its cyclodextrin inclusion compounds through a cellophane membrane, in aqueous suspension, at 25 °C (According to <46>, with permission of Acta Pharm.Suec.)

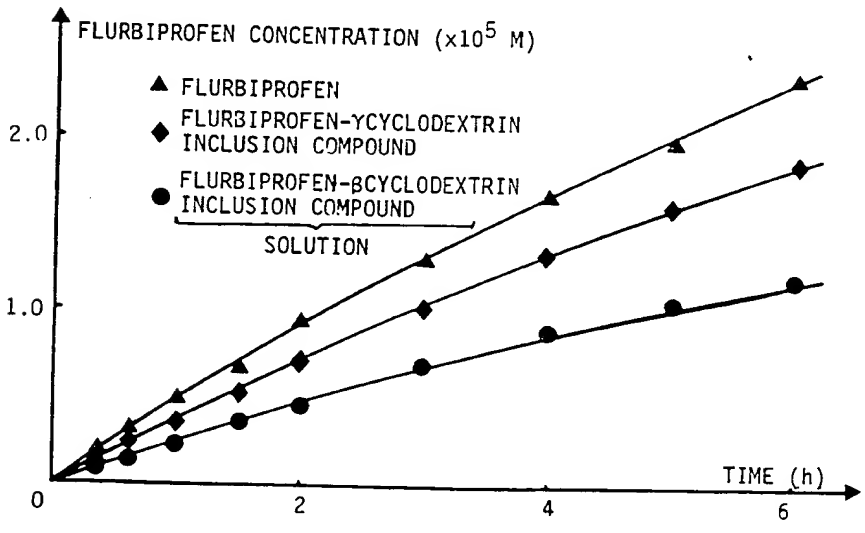

Figure 19 Permeation of flurbiprofen in the absence and presence of cyclodextrins through a cellophane membrane, in aqueous solution, at 25 °C (According to <46>, with permission of Acta Pharm.Suec.)

3.1.5 *Interfacial transfer*

Hoping to develop an experimental model imitating the *in vivo* absorption of inclusion compounds, Uekama <96> used an *in vitro* dissolution model S-L_W-L_O (solid phase/aqueous liquid phase/organic liquid phase) <83,95>. The model consists of a rotating disc dissolution apparatus with an organic phase (Figure 20). During the interfacial transfer

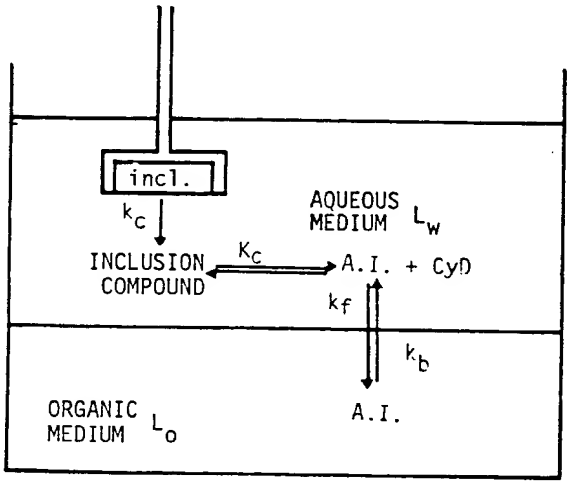

Figure 20 Schematic diagram of the S-L_W-L_O model
(According to <95>, with permission of Chem.Pharm.Bull.)

of the active ingredient following its dissoluton from the compressed tablet, the rates of change in concentration of the active ingredient in L_W and L_O is given by:

$$\frac{dC_W}{dt} = K \cdot (S_S - C_W) - k_f \cdot C_W + k_b \cdot C_O$$

$$\frac{dC_O}{dt} = k_f \cdot C_W - k_b \cdot C_O$$

where:

- C_W and C_O concentrations of active ingredient in L_W and L_O,
- k_f and k_b first-order rate constants for forward- and backward-interfacial transfer,
- K dissolution rate constant,

- S_s saturated concentration of active ingredient in L_w.

With pure substances, good correlation was observed between the theoretical concentration of product passing from the solid phase to the organic liquid phase and the experimental values. With inclusion compounds, the mechanism is much more complex, and the theoretical calculation of diffusion becomes problematic. However, the observation of the concentration in the organic phase always remains a good simulation of *in vivo* absorption for active ingredients resorbed by passive diffusion.

3.1.6 *In situ resorption*

In addition to the *in vitro* experimental model, Uekama developed and used an *in situ* experimental model S-L_W-*in situ* (solid phase/aqueous liquid phase/*in situ* <83,95,96>. This model consists in perfusing *in situ* a predetermined length of the small intestine of an anaesthetized rat or rabbit, by the aqueous dissolution liquid of the test product, as its dissolution proceeds, and regularly determining the active ingredient in the blood of the animal. Uekama showed that good correlation exists between the results of the study of *in vitro* interface transfer and *in situ* absorption.

A study of the same type was carried out by Szejtli and Szente <66>, who compared the absorption of indomethacin labelled with ^{14}C, alone or included in ß-cyclodextrin, in the small and large intestines of rats. In the case of indomethacin alone, 56% were absorbed in the small intestine and 6% in the large intestine. In the case of included indomethacin, absorption was 68 and 66% respectively.

3.2 *In vivo* investigations: improvement of bioavailability and modification of pharmacokinetics

Whatever the value of the foregoing techniques, they can merely offer the firm hope of an improvement in bioavailability, and this must be checked in animals and in humans.

3.2.1 *Oral administration*

The inclusion of an active ingredient in a cyclodextrin may reduce its bitterness <2,10> and, more interesting, any harmful side effects, such as the attack of the stomach mucous membranes by certain non-steroid anti-inflammatory substances. This is what happens with phenylbutazone included in ß-cyclodextrin, but is not observed with indomethacin or flufenamic acid <40>. The disappearance of irritation

caused by pirprofen on the mucous membrane of the throat is helped by inclusion in β-cyclodextrin <15>.

With respect to bioavailability, an improvement is usually observed if the inclusion of an active ingredient has already improved its dissolution. Not only is the blood concentration higher, with its peak occurring sooner, but the area below the curve (plasma concentration/time) is also larger. These results can be obtained, for example, after the oral administration of the following inclusion compounds: digoxin γ-cyclodextrin in the dog <79,81>, spironolactone β- or γ-cyclodextrin in the dog <53>, phenytoin β-cyclodextrin in the dog <77>, flurbiprofen β- or γ-cyclodextrin in the rabbit <46>, acetohexamide β-cyclodextrin in the rabbit <88>, diazepam γ-cyclodextrin in the rabbit <89>, ketoprofen β-cyclodextrin in the dog <41>, ketoprofen, ibuprofen and flufenamic acid β-cyclodextrin in the rabbit <41>, indomethacin β-cyclodextrin in the rat <66> (but no favourable effect is observed in the rabbit <41>), and allobarbital, amobarbital, barbital, pentobarbital or phenobarbital β-cyclodextrin in the rabbit <24>.

A similar result is obtained by the oral administration to humans of the inclusion compounds salicylic acid β-cyclodextrin <9> or prednisolone β-cyclodextrin <94>. These studies also reveal the value of the oral administration of freeze-dried drugs <52,77>. In some cases, the improvement in bioavailability caused by inclusion is such that a reduction in the dose administered can be considered. This applies in particular to the inclusion digoxin γ-cyclodextrin (1:4) <79,81>, for which the area under the curve obtained with the inclusion compound containing 50 µg of digoxin is higher than the one obtained with 100 µg of pure digoxin (Figure 21). Furthermore, the inclusion

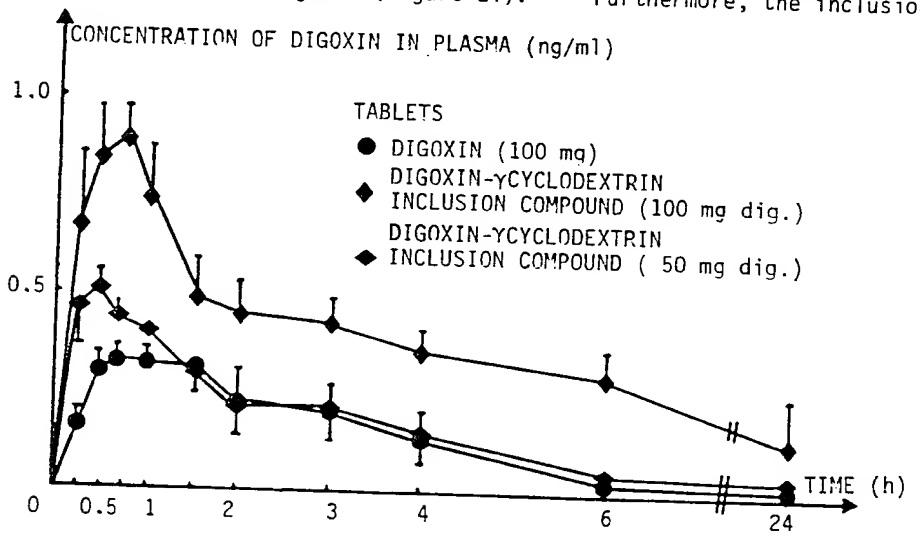

Figure 21 Plasma levels of digoxin following the oral administration of tablets containing digoxin or 1:4 digoxin γ-cyclodextrin inclusion compound to dogs
(According to <81>, with permission of the copyright owner, the American Pharmaceutical Association)

compound 1:4 gives an eightfold increase in the molecular weight of the active ingredient, facilitating the handling of low doses of the product, and leading to more regular dosage of the units prepared.

It may sometimes be advantageous to administer an additive at the same time as the inclusion, to improve its *in vivo* effectiveness. This applies in particular to cinnarizine, whose inclusion in β-cyclodextrin increases its solubility at pH 3.0 to 6.8, but does not change its bioavailability. Accordingly, the stomach pH must be adapted to better dissolution, and this is done by the simultaneous administration of $NaHCO_3$ <76>. For the same product, the administration of a competing agent, such as DL-phenylalanine, also proves to be interesting. After oral administration, the dissociation of the inclusion cinnarizine β-cyclodextrin is facilitated by the presence of phenylalanine, which tends to supplant the cinnarizine in the cyclodextrin, causing the absorption of cinnarizine, now in the molecular state, to occur rapidly <75> (Figure 22).

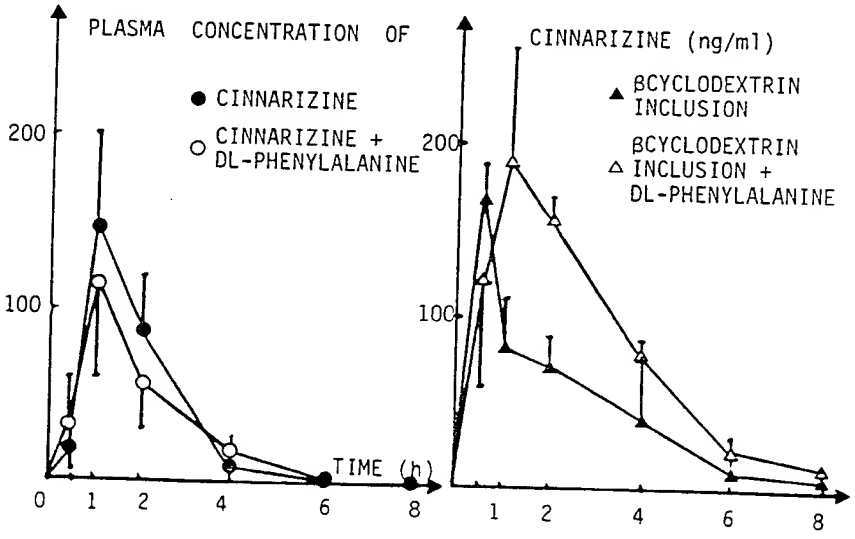

Figure 22 Plasma concentration of cinnarizine after oral administration to dogs of cinnarizine (50 mg) and the inclusion compound of cinnarizine and β-cyclodextrin (equivalent to 50 mg of cinnarizine) with or without 2 g of DL-phenylalanine
(According to <75>, with permission of the copyright owner, the American Pharmaceutical Association)

Improved bioavailability should normally be reflected by an increase in the therapeutic effect. This was observed by Koizumi, who investigated five barbiturates (phenobarbital, pentobarbital, amobarbital, allobarbital and barbital) <25>. Their 50% effective dose (ED_{50}) was actually reduced to varying degrees by inclusion in β-cyclodextrin.

From these results, Szejtli <63> shows that the hypnotic activity of the inclusion compounds (reduction of the 50% effective dose) is in good correlation with the effect of inclusion on the corresponding barbituric solubility (Figure 23).

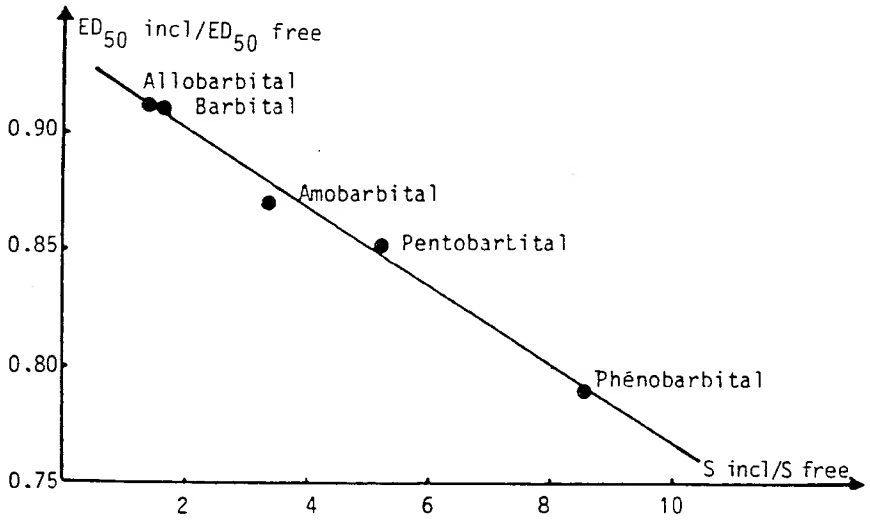

Figure 23 Correlation between enhancement of solubility (S incl/S free) and enhancement of hypnotic activity (i.e. reduction of ED_{50}) by ß-cyclodextrin inclusion of barbiturates
(According to <63>, with permission of Stärke)

By administering 1.2 to 2.5 50% effective doses of barbiturates, alone or included (depending on the short or long sleeping effect), Koizumi <25> shows that the sleeping lags are shortened by the inclusion, with the exception of barbital (Table 9). This might be due to the better solubility of inclusion compounds and their faster resorption. Once more, with the exception of barbital, sleeping times are always significantly enhanced by inclusion.

Therapeutic improvements are also observed by Szejtli <8,61,62> by the administration of vitamin D_3 to rats.

Greater increases in urinary volume in the rat were observed with the administration of spironolactone included in ß-cyclodextrin than with spironolactone alone <5> (Figure 24).

Table 9

Solubilities, stability constants, 50% effective doses, and sleeping times of mice for barbiturates and their inclusion compounds in β-cyclodextrin (According to <24>, with permission of Chem.Pharm.Bull.)

product	constants		50% effective dose	dose (μmol/10 g)	sleeping	
	S ($mol \cdot 10^2$)	K (mol^{-1})			lag (min)	time (min)
phenobarbital β-cyclodextrin	7.0	787	2.91	4.5	27.0	241.0
phenobarbital	0.8		3.72		75.7	122.0
pentobarbital β-cyclodextrin	3.8	619	0.91	2.7	5.0	63.9
pentobarbital	0.7		1.07		14.3	18.0
amobarbital β-cyclodextrin	1.4	562	3.00	4.9	6.7	86.3
amobarbital	0.4		3.44		20.1	49.7
allobarbital β-cyclodextrin	1.9	98	1.61	2.9	12.6	136.1
allobarbital	1.2		1.76		23.3	93.6
barbital β-cyclodextrin	9.8	62	8.02	10.9	31.6	128.4
barbital	5.3		8.73		31.3	122.4

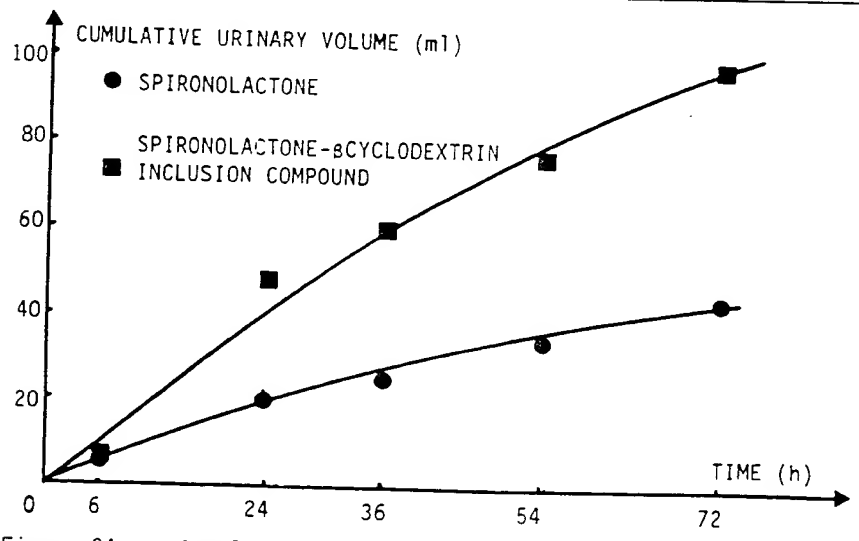

Figure 24 Cumulative urinary volumes after oral administration to the rat of 50 mg/kg of spironolactone alone or included in β-cyclodextrin, Force-feeding at t = 0, 24 and 36 h (According to <5>)

3.2.2 *Rectal administration*

The rectal administration of suppositories containing active ingredients, alone or in cyclodextrin inclusions, is often reflected by greater bioavailability <46,57,88>. In actual fact, it appears that the type of excipient has a significant effect on the bioavailability of the active ingredient itself or of the inclusion compound <19>. This was observed with phenobarbital included in β-cyclodextrin, combined with Witepsol S 55 or with Macrogol (Figure 25). The inclusion

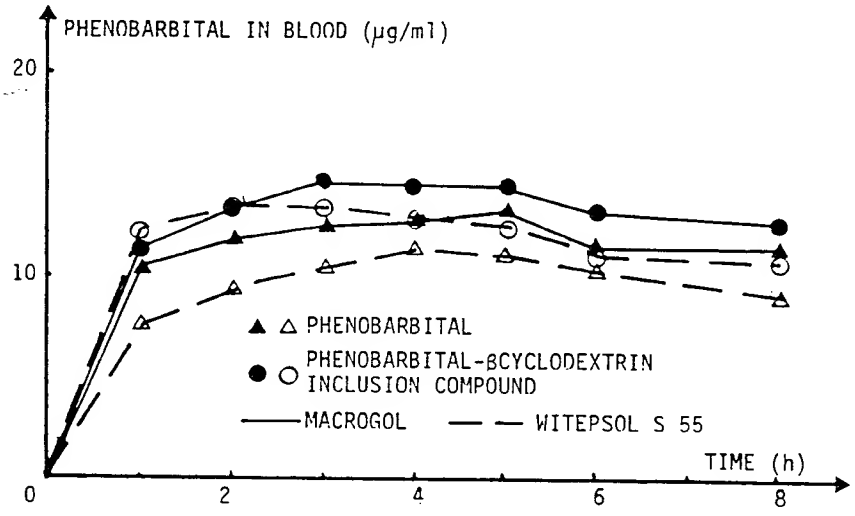

Figure 25 Blood levels of phenobarbital following rectal administration of phenobarbital β-cyclodextrin inclusion compound and phenobarbital in Macrogol or Witepsol S 55 suppositories to rabbits (According to <19>, with permission of Chem.Pharm.Bull.)

compound gives high blood levels when associated with Witepsol S 55 or Macrogol. On the other hand, phenobarbital alone leads to an appreciably lower blood level from its association with Witepsol than with Macrogol. In this case, it should be noted that cyclodextrin tends to delay the absorption of phenobarbital in the rectum, so that the higher blood levels with the inclusion compound can essentially be attributed to a faster release of this compound (hydrophilic) than of the phenobarbital, based on the excipients employed <19>.

3.2.4 *Ocular administration*

Very few tests have so far been conducted on this method of administration. However, it is worthwhile noting the possibility of reducing local irritation caused by various products <29> and especially flurbiprofen <30>, when they are included in β-cyclodextrin. It also appears that the inclusion of sodium sulphacetamide in β-cyclodextrin improves its release from an ophthalmic ointment <54>.

3.2.5 *Parenteral administration*

While the non-toxicity of cyclodextrins by oral administration appears to be highly probable <60>, this cannot be said of parenteral administration. Tests have nevertheless been conducted by this method in animals.

Nagai <38> administered hexobarbital in the presence of α-, β- and γ-cyclodextrins to mice and rats by intravenous and intraperitoneal administration. A significant change in the pharmacokinetics of the product resulted from the presence of cyclodextrins. Ten and twenty minutes after intravenous administration, the following effects were observed in comparison with hexobarbital alone: higher blood and kidney concentrations, lower brain and liver concentrations, shorter sleeping time, and prolonged latency period before induction of sleep.

4 CONCLUSION

The possibilities of using cyclodextrins in the pharmaceutical industry seem to be numerous. The vast number of papers and patents dealing with them testifies to the great interest they have roused.

In the beginning, it seemed that their only value lay in solid oral dosage forms. More recently, works report new directions of use: the rectal, dermal and ocular routes. This apparent change can be explained by the fact that, in various countries, such as France, cyclodextrins are not yet allowed as alimentary adjuvents, due to the long chronic toxicity studies, which, for the moment, are not complete. The interest revealed by works undertaken on products administered orally, namely improvement in stability and solubility, appeared to be transposable to other pharmaceutical forms for which the toxicity studies are not so demanding.

However, the various investigations carried out in the pharmaceutical industry, as well as in the parapharmaceutical industries, seem to hold promise for impending industrial exploitation.

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